

FIGURE 1: CTLA-4 VLD-SPECIFIC OLIGONUCLEOTIDES

OLIGONUCLEOTIDE	NUMBER	SIZE	SEQUENCE	FEATURES
For 5' CTLA-4 amplification	3553	54	TTATTACTCGCGGCCAGCCGCAATGGCCGCAATGGCACGTGGCCAGCCTGCT	forward primer + SfiI site
5023				
5671				
For 5' CTLA-4 amplification	3445	60	TTATTACTCGCGGCCAGCCGCAATGGCCGCAATGGCACGTGGCCAGCCTGCTGGTA	forward primer + SfiI site
For 5' CTLA-4 amplification	5443	45	TCTCACAGTGCACAGGCAATGGCACGTGGCCAGCCTGCTGGTA	forward primer + ApaI site
For 5' CTLA-4 amplification	4851	39	TCTCACAGTGCACAGGCAATGGCACGTGGCCAGCCTGCT	forward primer + ApaI site
For 5' CTLA-4 amplification	5467	43	GGCAGCCGGCCGAATTCGCAATGGCACGTGGCCAGCCTGCTG	forward primer + EcoRI site
For 5' CTLA-4 amplification		60	GCACCAATACGACTCTCTATAGGAAAGAGACCACTGGACCGTGGCGCCAGCCTGCTGCG	ribosomal display
4316		42	ATCTGGCGCGCTACATAAATCTGGGTACCGATTGGCGATTGCC	reverse primer + NotI site
5022				
5670				
For 3' CTLA-4 amplification	4486	66	GCCTGAATCTGTATCAGTGAATGGTGAATGGTGGCCGCTCAGATCTTGGGCACGGTTCCTGG	ribosomal display 1
For 3' CTLA-4 amplification		51	GCCTTGGGCGGGAGATGCTCTGCTTCAGTGGCGAGGCGCAGGTCTGTGTG	ribosomal display 2
For 3' CTLA-4 amplification		49	CGAGGCCAGGTCTGTGTGTGCTACCGCTTCACCTGAACTCTCCCGGAG	ribosomal display 3
For 3' CTLA-4 amplification		51	CGTGAACCTCTCCCGGAGTTCACAGTCACTCTCCAGATGCTGGCCCTCACCC	
4585		84	AGCTTTGTGTGTGAGTATGCAGCTGGCTGTGCAAGATTTCTTCTTGGTAGACTTTCACATCCTGTGGCAGCTpAgg	forward primer + Som
4586		84	TCCGGGTGACA	reverse primer + Som
4835		75	CTGGGTACCGTTGCCGATGCCACAGGATGTGAAAAGTCTTCCAGAGAAATTCCTTGCAGCCAGCCTCCACCTTGCAGATGTAGAg	
For CDR1-somatostatin				
For CDR3-somatostatin				
For CDR1-som-randomisation				

Figure 1 (con't'd)

Figure 1 (cont'd)

CTLA-4 codon-change	5599	66	CC	CAGACCCCTggATggTCAGgTTCACCTggTTACCGCTggAggTgCCggTgCAgATggAATCATCCAg
CTLA-4 codon-change	5597	57		CACTTTgCAgATgTACAgACCggTATCCATggCACgCAGACCCTggATggTCAggTT
CTLA-4 codon-change	5606	66		CAGCCATgACCgCATTCgTAAATAgACgCATAgATggTgCTATCCACTTTgCAgATgTACAgACC
CTLA-4 codon-change	5607	69		CTgggTACCgTTgCCgATgCCAGAAATCgTAgCCATAgCCACCggTggACAggCCATgACCgCATTCgTA
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¹ All oligonucleotides are described 5' to 3'.

² N represents combination of the four nucleotides

Figure 1 (cont'd)

Figure 2: Polynucleotide sequence of complete cDNA encoding human CTLA-4 and polypeptide sequence of the VLD of human CTLA-4.

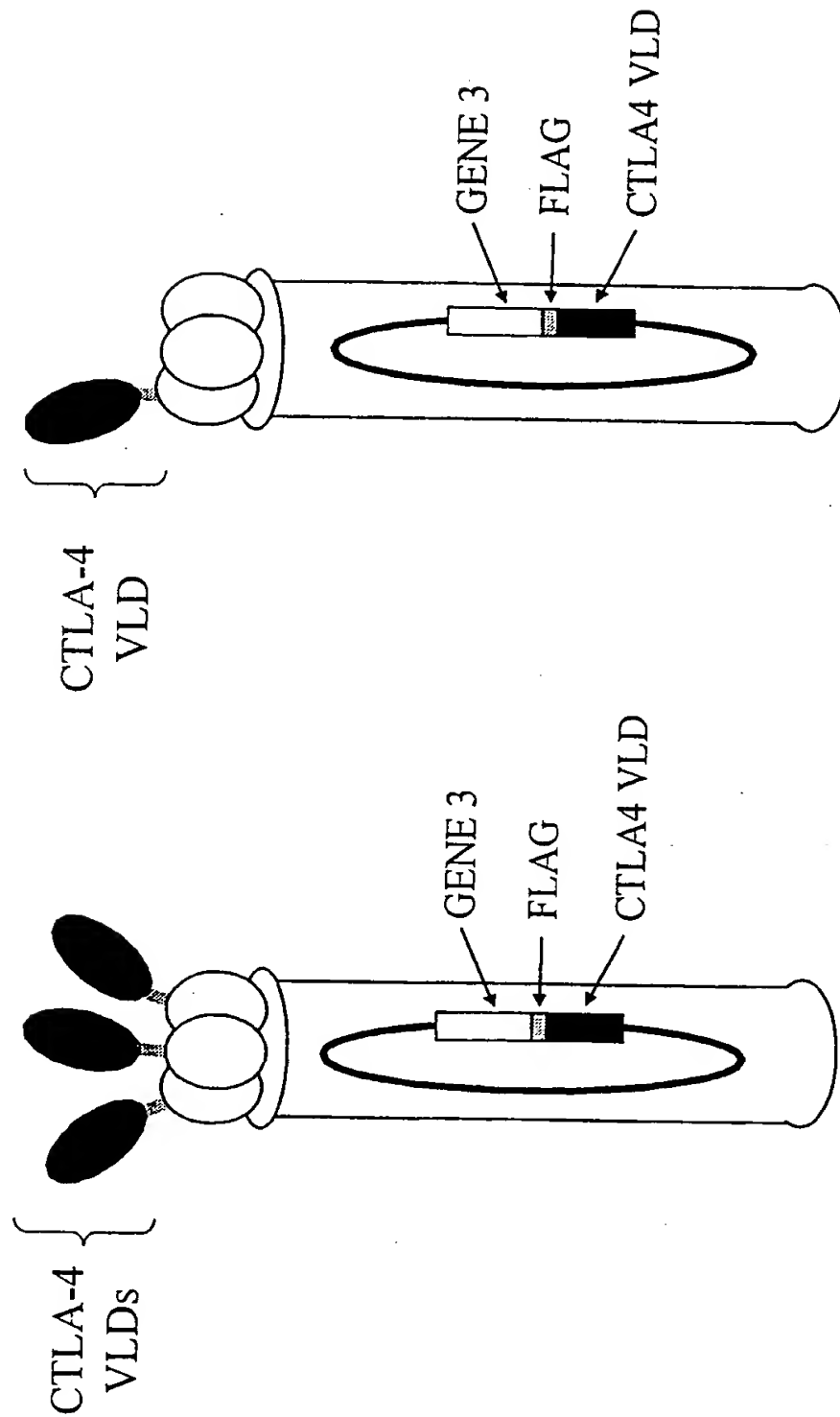
A. Polynucleotide Encoding Human CTLA-4 cDNA

ATGGCTTGCC TTGGATTTC A GGGCACAAG GCTCAGCTGA ACCTGGCTGC CAGGACCTGG CCCTGCACTC TCCTGTTT... 80
TCTTCTCTTC ATCCCTGTCT TCTGCAAGC AATGCACGTG GCCCAGCCTG CTGTGCTACT GSCCAGCAGC CGAGGCATCG... 160
CCAGCTTTGT GTGTGAGTAT GCATCTCCAG GCAAGGCCAC TGAGGTCCGG GTGACAGTGC TTCGGCAGGC TGACAGCCAG... 240
GTGACTGAAG TCTGTGCGGC AACCTACATG ACGGGGAATG AGTTGACCTT CCTAGATGAT TCCATCTGCA CGGGCACCTC... 320
CAGTGGAAAT CAAGTGAACC TCACTATCCA AGGACTGAGG GCCATGGACA CGGGACTCTA CATCTGCAAG GTGGAGCTCA... 400
TGTACCCACC GCCATACTAC CTGGGCATAG GCAACGGAAC CCAGATTAT GTAATTGATC CAGAACCCTG CCCAGATTCT... 480
GACTTCTCC TCTGGATCCT TGCAGCAGTT AGTTCGGGGT TGTCTTTT TAAGCTTTCTC CTCACAGCTG TTTCTTTGAG... 560
CAAAATGCTA AAGAAAGAA GCCCTCTTAC AACAGGGGTC TATGTGAAA TGCCCCAAC AGAGCCAGAA TGTGAAAAGC... 640
AATTTCAGCC TTATTTTATT CCCATCAATT GA ... 672

B. Polypeptide Sequence of Human CTLA-4 VLD

1	10	20	30	40	50	60	70	80
AMHVAQPAV	LASSRGIASF	VCEYASPGKA	TEVRVTVLRLQ	ADSQTEVCA	ATYMMGNELT	FLDDSICTGT	SSGNQVNLTI	
90	100	110	115					
QGLRAMDTGL	YICKVELMYP	PPYVLGIGNG	AQIYV					

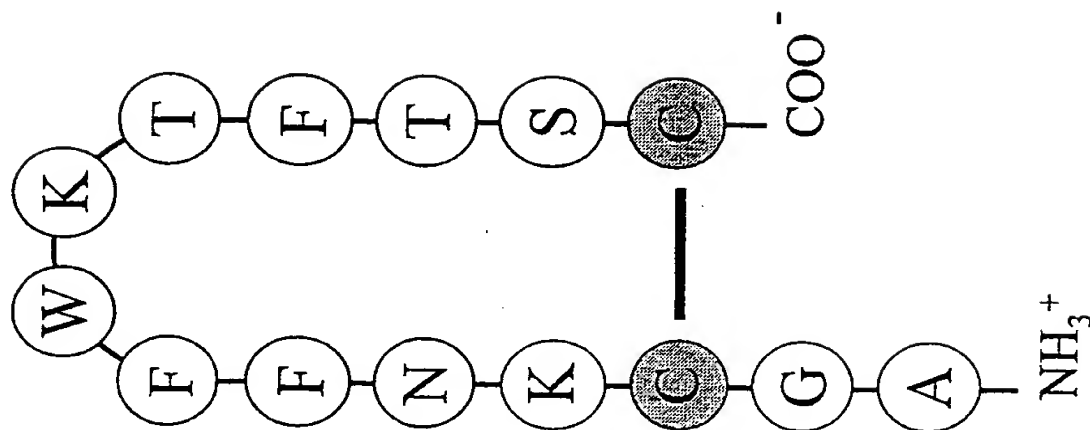
Figure 3: Phage display of CTLA-4 VLD STMs



PHAGEMID GENE 3 FUSION

PHAGE GENE 3 FUSION

Figure 4: Schematic representation of the somatostatin polypeptide.



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Figure 5: Comparison of the HPLC profiles of affinity purified CTLA-4 VLD and CTLA-4-Som3 STM

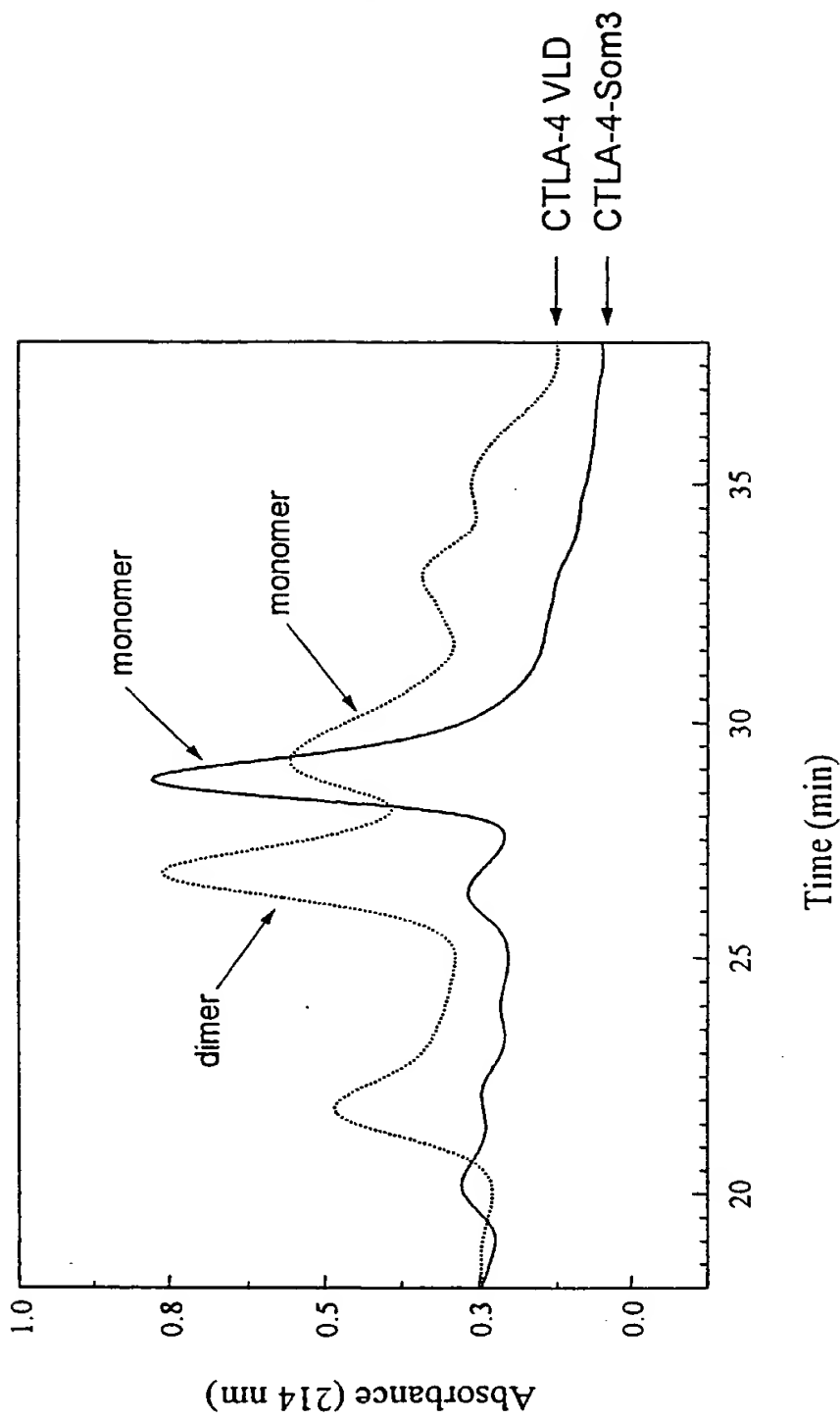


Figure 6: Schematic diagram of CTLA-4 VLD CDR loop replacements.

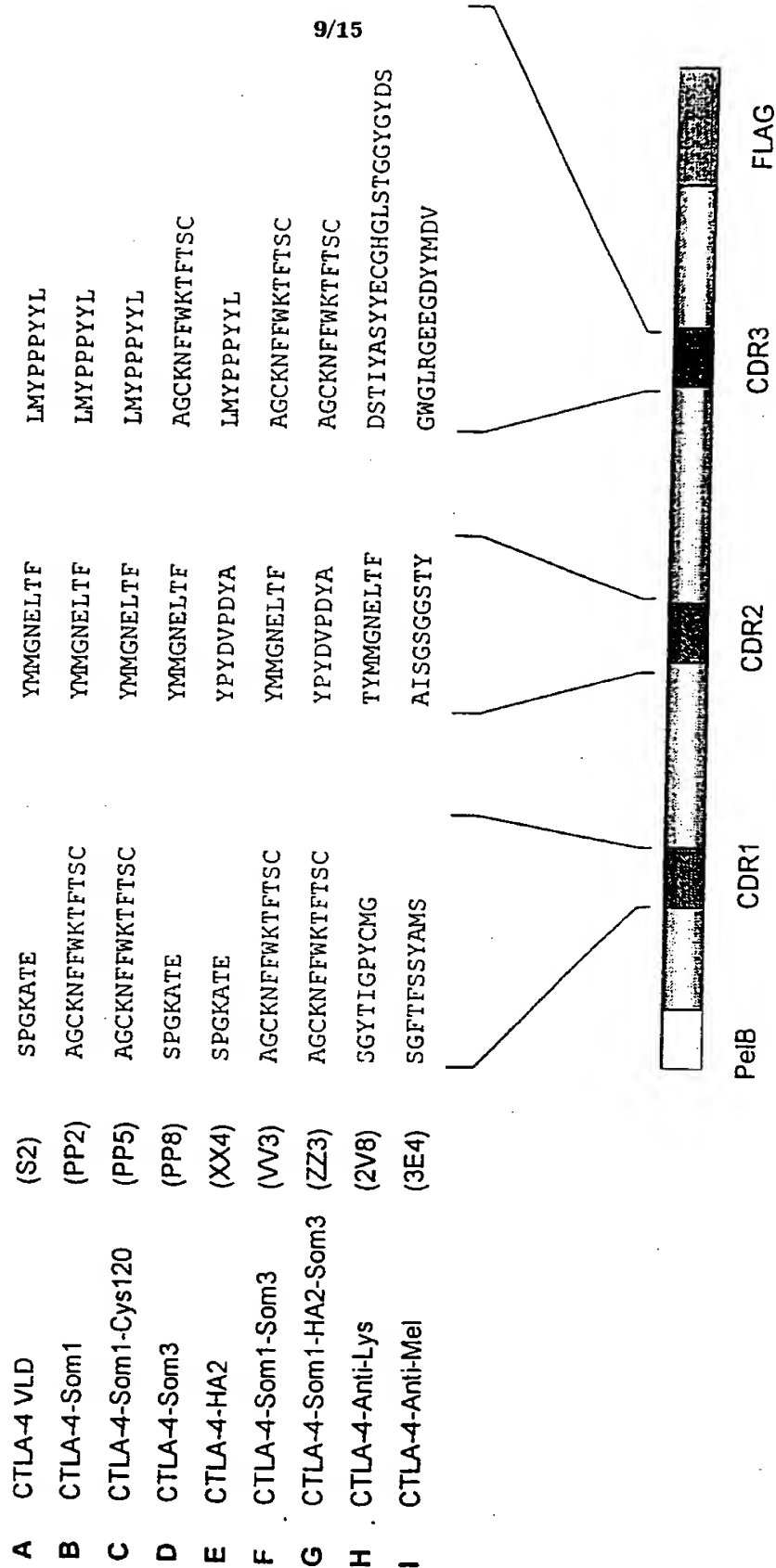


Figure 7: HPLC profiles of affinity purified recombinant CTLA-4 STMs

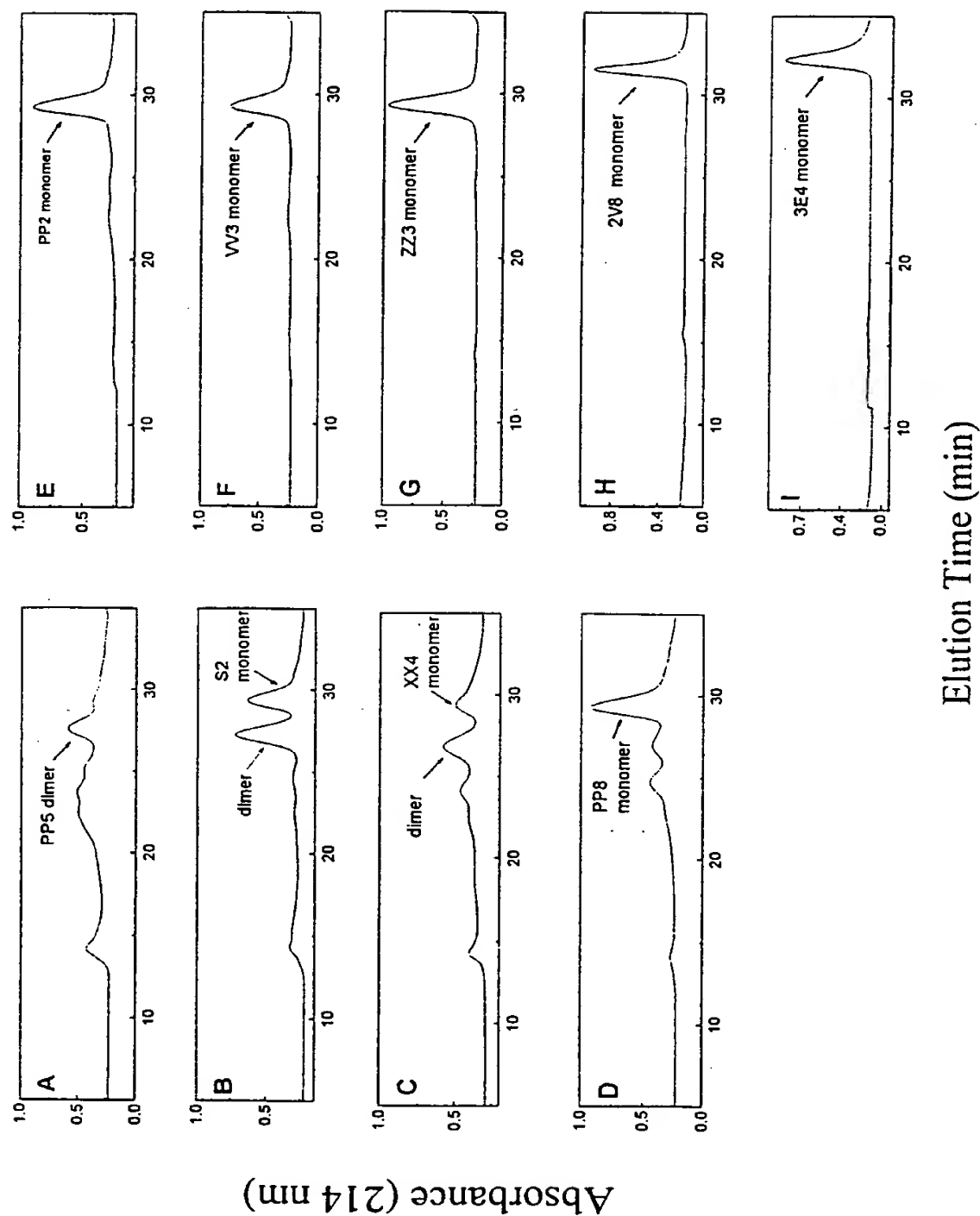


Figure 8: Comparison by size exclusion HPLC analysis of affinity purified CTLA-4 VLD STM constructs synthesised using bacterial expression vector pGC or pPOW.

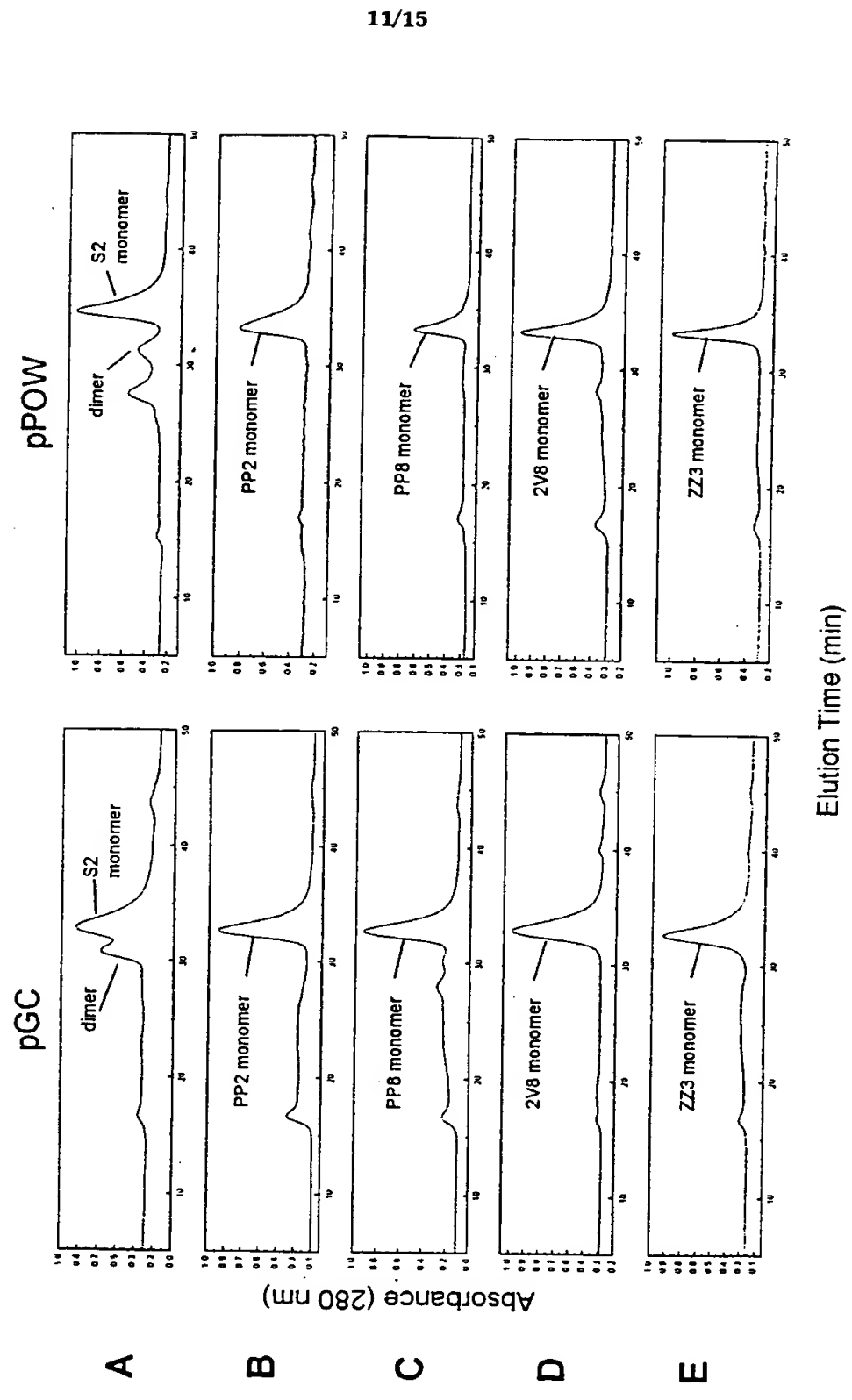


Figure 9: Size exclusion HPLC analysis of affinity purified CTLA-4 VLD STMs: Effect of Freeze/Thaw upon protein

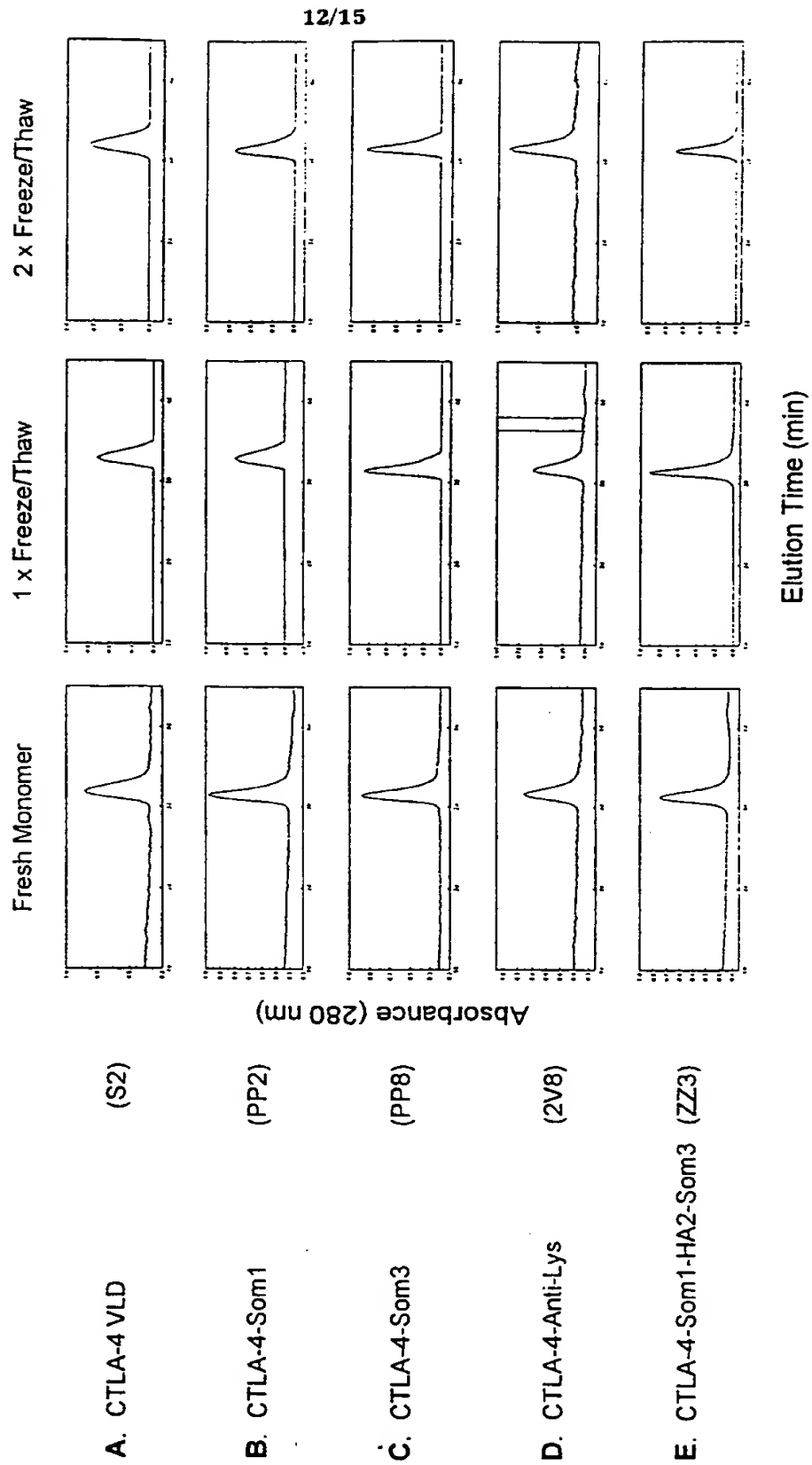


Figure 10: Lysozyme binding characteristics of CTLA-4 anti-lysozyme construct 2V8

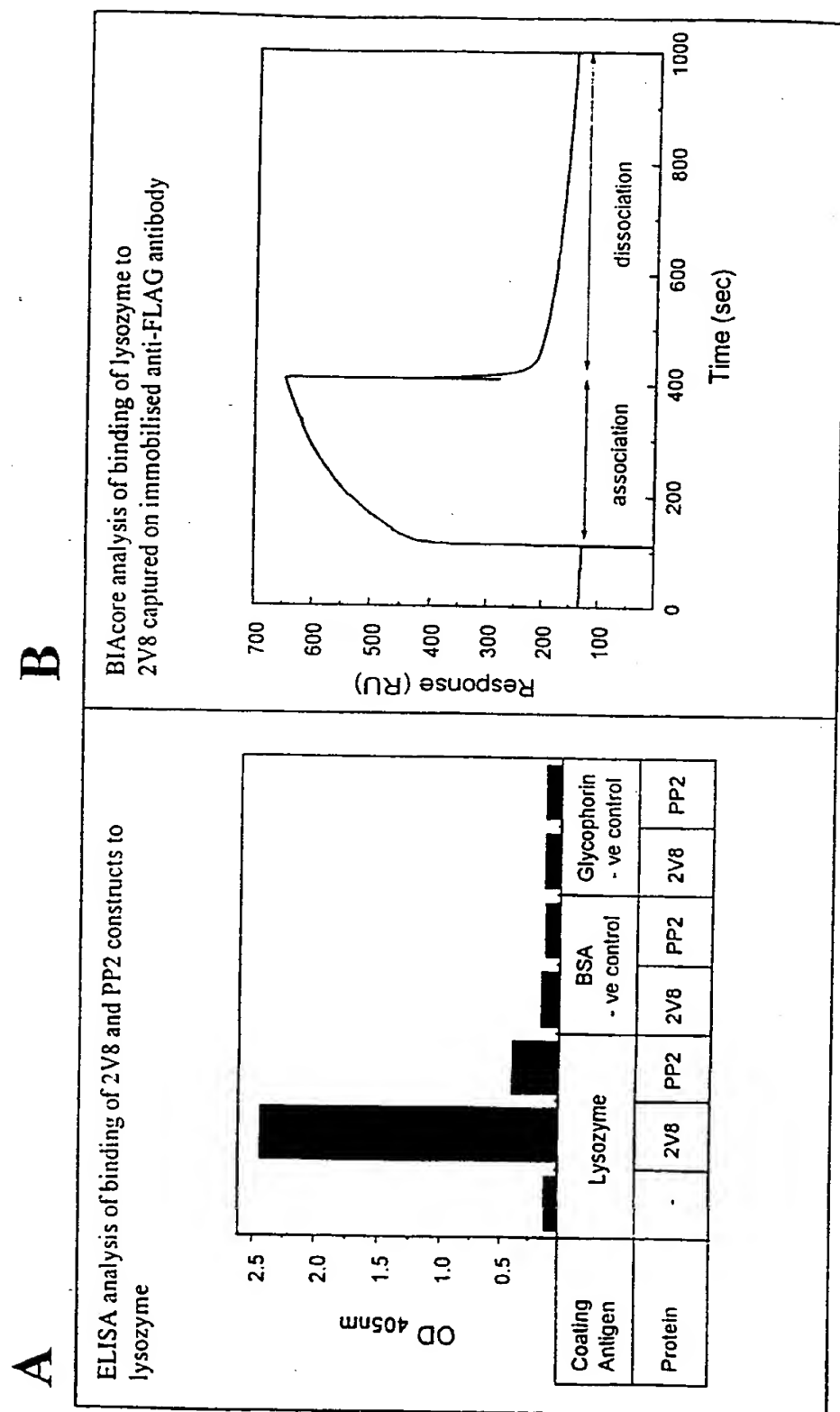
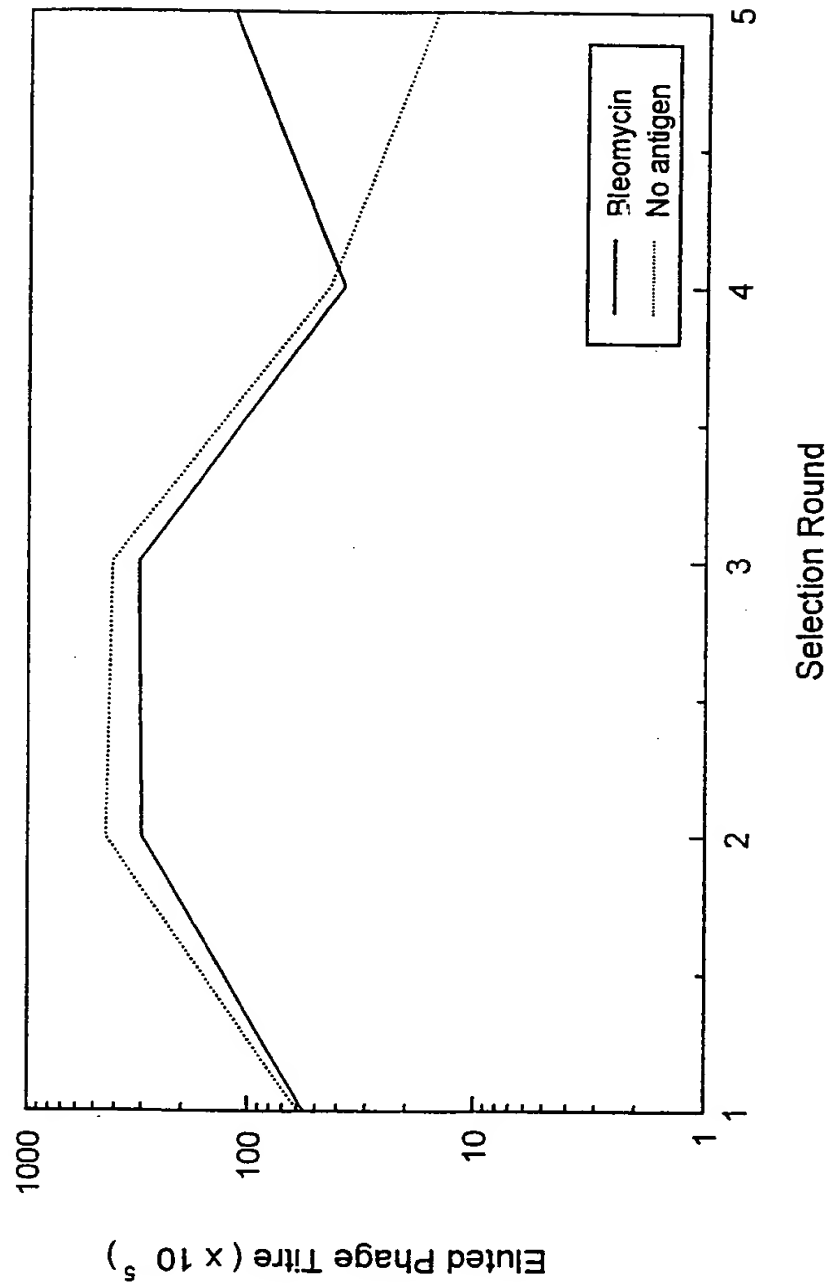


Figure 11: Screening of CTLA-4 VLD Phagemid Library on Immobilised Sh Bleomycin



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Figure 12: Screening of CTLA-4 VLD libraries in solution.

